

Fecal Elastase-1 Levels in Preterm Infants with Bronchopulmonary Dysplasia

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Abstract

Bronchopulmonary dysplasia (BPD), defined as the need for oxygen at 28 days of life, develops in preterm infants as a result of ventilatory strategies used to manage acute respiratory illness. The incidence rate of BPD is increasing as more and more extremely preterm infants survive acute respiratory illness. Inadequate growth is the most common morbidity affecting these infants, with attained weights well below the 10th percentile. One area in need of further research is the examination of the ability of the preterm infants with BPD to digest provided nutrients. The only study to date found that these infants have decreased pancreatic activity and increased fecal losses of nutrients, excreting 21.4% of fat intake and 13.5% of protein intake. This is an important finding given that the standard of care is to increase nutrients to promote growth. The purpose of this exploratory study was to examine pancreatic enzyme activity in preterm infants with BPD. Data were collected on preterm infants on full enteral nutrition at approximately 33 weeks post-conceptual age. Fecal elastase-1 levels ($\mu\text{g/gm}$ of stool) were measured and compared to the expected normal value of 200 $\mu\text{g/gm}$ of stool. Enzyme-linked immunosorbent assay (ELISA) was used to determine fecal elastase-1 levels. In our laboratory, the coefficient of variation for assays is less than 10%. To date, there has been very limited examination of the ability of preterm infants with BPD to digest the nutrients provided. The anticipated findings from this proposed project should provide a better understanding of digestion and provide the foundation for further study of gastrointestinal function in preterm infants with BPD.

Chapter 1

Introduction

Premature birth is a major problem affecting the infant population. In 2006, there were 542,893 preterm births in the United States, representing 12.8% of all live births (National Center for Health Statistics, final natality data). Majority of these preterm infants develop Respiratory Distress Syndrome (RDS), which is the most frequently observed severe acute illness in this population (Martin et al., 2002). Within this class, Bronchopulmonary Dysplasia is the most common form of chronic infant lung disease and the most common cause of morbidity and death among these preterm infants. Many advances in the treatment of preterm infants with BPD have been discovered and implemented and thus, the survival rates of these infants have increased. Several examples of new these new treatments include an increase in the usage of glucocorticosteroids, exogenously supplied surfactant, and technologically advanced ventilator strategies (Coalson, 2003). Despite the increased survival rate of the preterm infants with BPD, the incidence rate of BPD still remains high. Thus, the search for new technologies to decrease the occurrence rate of BPD and to improve the outcomes continues to be a pressing issue.

Growth is one of the most essential therapies for preterm infants with BPD. This is because lung growth and development are closely related to the rate of somatic growth. However, this is problematic because inadequate growth and nutrition intake is one of the common morbidities affecting the preterm infants with BPD (Karn & Steward, 2005). While many health care providers recommend increasing the macronutrient caloric intake of these infants in order for them to grow, research suggests that this may not be the best

solution (Carroll, Slobozian, & Steward, 2005). The reason for this is that very little is known about the digestive and absorptive ability of premature infants with BPD (Campeotto et al., 2007). Thus, this could indicate that preterm infants with BPD are not able to properly absorb or digest macronutrients and that calories are being lost throughout the digestive process. Thus, the preterm infants with BPD are unable to maintain the same growth rate as a stable preterm infant of the same age without BPD (Karn & Steward, 2005).

Since more research is needed in this area, fecal pancreatic enzyme elastase-1 (FE-1) levels will be examined across time in preterm infants with BPD. FE-1 was the chosen pancreatic enzyme because of its ability to remain stable throughout the digestive and absorptive processes (Kori, Maayan-Metzger, Shamir, Sirota, & Dinari, 2003). Thus, the FE-1 concentration level determined from the collected stool samples will be used to help determine the exocrine pancreatic function and capacity of the preterm infants with BPD.

Within this study, several aspects of the preterm infant's nutrition, weight gain, and FE-1 levels will be examined. The FE-1 levels of the preterm infants with BPD will be examined and compared to the normal FE-1 level, that is considered to be equal to or greater than 200 $\mu\text{g/gm}$ of stool. The hypothesis is that preterm infants with BPD will have inadequate FE-1 levels and thus, will fall below the normal range of 200 $\mu\text{g/gm}$ of stool. By conducting this research, a better understanding of the digestive and absorptive abilities of these preterm infants with BPD will be able to be determined.

Chapter 2

Review of Literature

A. BPD

With the improved survival rate of smaller, preterm infants that has been seen in recent years, BPD has become a common morbidity among this population. It is estimated that 77% of extremely low birth weight infants develop BPD (Ehrenkranz et al., 2005). In the United States alone, the annual cost to care for preterm infants with BPD is estimated to be around \$2.4 billion (National Institute of Health, 1998). BPD is defined as the requirement of supplemental oxygen and/or mechanical ventilation support at 28 days of life (Ehrenkranz et al., 2005). This support results in treatment-induced injury, which causes an inflammatory cascade and results in hypoalveolarization (Coalson, 2003). BPD is a disruption in the normal lung development with the main pathophysiological changes being alveolar simplification and impaired angiogenesis of the lungs (Thebaud & Abman, 2007). Children with a history of BPD also have an increased risk of developing developmental disorders, lower pulmonary functioning, growth retardation, and academic difficulties in school (Anderson & Doyle, 2006; Eber, 2001). In addition, this chronic disease can have a major impact on the daily lives of the families caring for these preterm infants with BPD (Kobaly et al., 2007). While more data is being collected and new strategies are being implemented, the incidence of BPD continues to remain high (Wadhawan et al., 2007).

B. BPD and Growth

Failure to grow in infants with BPD is a major issue. Growth accounts for the largest amount of energy expended in infants (Pridham et al., 2007). Inadequate

nutritional intake, poor intestinal absorption, and increased energy expenditure are all factors that can contribute to this poor growth rate (Boehm et al., 1996). The goal weight gain for preterm infants is to be at approximately the same rate as the expected intrauterine rate of growth of a fetus. This rate is 15 g/kg/day (American Academy of Pediatrics, 1998). However, if the preterm infant is not able to maintain a growth rate of 15 g/kg/day or higher, it is projected that the weight gain of the infant will be five to six weeks behind that of the growing fetus (Heird, 2001). Furthermore, even if the goal growth rate is able to be met, it is extremely hard to maintain this growth rate because of the preterm infant's physiologic immaturity.

Furthermore, while a 10% weight loss following birth is expected in infants, the low weight preterm infants are expected to lose between approximately 15%-20% of their birthweight. By 36 weeks postconceptional age, 99% of these infants have fallen below the 10th percentile for growth. (Carroll et al., 2005). This is important because it shows that the preterm infants with BPD develop a significant growth deficient that can be hard to overcome when taking into account all of the other contributing factors (Ehrenkranz et al., 1999).

In the longitudinal study performed by Boehm et al. (1996), several factor were examined to determine the degree to which each of these factors affected the growth rate in preterm infants with BPD. These factors included mean duodenal lipase and trypsin activity levels, mean total bile acid concentrations, fecal fat excretion, and nitrogen concentration levels in preterm infants. They examined these factors in preterm infants with BPD, preterm infants without BPD, and term infants, who were used as the control group. The lipase, trypsin, and bile acid concentration levels were obtained by inserting a

transpyloric tube into the duodenum and preprandially aspirating digestive fluid (Boehm et al., 1996). Stool samples were used to determine total nitrogen and total fat levels. The results of the Boehm study showed that preterm infants with BPD had a decrease in pancreatic enzyme sufficiency and limited fat absorption. They also showed that both of these factors may result in inadequate weight gain and an insufficient growth rate in the preterm infants.

The small amount of research that has been conducted in this area shows that the preterm infants with BPD have an increased energy expenditure that may contribute to their inability to gain weight at the same rate as the expected intrauterine growth rate. In order for growth to occur, the caloric intake must exceed the amount of energy expended (Carroll et al., 2005). Energy expenditure can be defined as the physiologic processes that fuel growth, development and other life processes. (Pridham et al., 2007). In the longitudinal study performed by Pridham et al. (2007), several biobehavioral variables that affect energy expenditure levels in preterm infants were examined. The researchers examined the infants activity level, caloric intake, biological maturity level, history of lung disease, and daily weight gain to determine the effects these factors had on the infants energy expenditure levels. To determine the amount of energy spent with each activity, a metabolic gas monitor indirect calorimeter was used. The results showed that a lung disease history, mean activity levels, and time were all important factors in determining energy expenditure in these preterm infants (Pridham et al, 2007). Thus, the more energy these preterm infants expend, the less they have available for growth. By determining the factors that have the largest impact on energy expenditure levels,

measures can be taken to minimize this energy loss, allowing more energy to be directed toward the preterm infant's growth processes.

C. Nutrition

Nutrition is one of the factors that plays a major role in growth and development. A significant portion of poor growth rates in preterm infants with BPD can be attributed to inadequate nutrition (Ziegler, Thureen, & Carlson, 2002). Thus, nutritional support has become an essential component in the provision of care for these infants (Embeleton, Pang, & Cooke, 2001). Research has shown that in the first few weeks post-birth, the preterm infants suffering from BPD receive significantly fewer calories than the stable preterm infants. (deRegnier, Guilbert, Mills, & Georgieff, 1996). Furthermore, the preterm infants received below the daily caloric and protein recommendations, which were 120 kcal/kg/day and 3 g/kg/day respectively (Ziegler et al., 2002). This resulted in a significant energy and protein deficiency and a cumulative energy deficiency of 598 kcal/kg (Karn & Steward, 2005). Thus, there is a limited amount of protein and fat available for the deposition of new body tissue. Because of this, the ability of the infant to grow is limited. (Carroll et al., 2005).

Another problem that is being faced by healthcare providers initiating nutritional support in preterm infants with BPD is the lack of universal nutritional guidelines. This results in nutritional practices that vary greatly in regards to the initial introduction of parenteral nutrition, the amount of macronutrients initiated, and the pace at which to advance these macronutrients (Carroll et al., 2005). One recommendation by Ziegler (2002) is to introduce enteral nutrition earlier in order to stimulate the maturation process

of the gastrointestinal tract. By doing this the infant will have a quicker advancement to full enteral feedings.

It is clear that inadequate nutrition related to growth deficiency is a large problem affecting preterm infants with BPD. While it is not clear how undernourished these infants actually are, it is hypothesized that the preterm infants with BPD require higher intake levels of protein, fat, and calories than stable preterm infants in order to establish the recommended growth rates (Karn & Steward, 2005).

D. BPD and Nutrient Digestion and Absorption

Providing adequate nutrition to preterm infants with BPD not only depends on the amount of calories, protein and fat provided, but also on the infants' physiologic ability to digest and absorb these nutrients. BPD is associated with a chronic inflammatory response and metabolic/respiratory acidotic state (Coalson, 2003). This makes it difficult for the body, including the gastrointestinal tract, to function properly. Since minimal research has been conducted in this area, the functionality of the GI system in preterm infants with BPD is an area in which little is known.

There is some evidence, however, that indicates that preterm infants with respiratory disease have decreased pancreatic activity levels. The most accurate way to determine pancreatic enzyme activity involves using a transpyloric tube to aspirate the enzymes in the duodenum. However, this method is highly invasive, costly and can be extremely stressful to the already unstable infants. Because of this, FE-1 is used as a marker to determine the exocrine pancreatic function. FE-1 is the most common pancreatic enzyme used to measure the pancreatic secretory capacity and exocrine function in preterm infants with BPD (Kori et al., 2003). FE-1 is considered to be a

highly specific and sensitive marker for pancreatic exocrine function and is able to remain stable throughout the intestinal transit (Campeotto et al., 2002; Kori et al., 2003). In addition, the FE-1 concentration is independent of any of the other exogenous pancreatic enzymes within the endocrine system (Nissler, Von Katte, Huebner, & Henker, 2001). Because of all of these factors, FE1 is thought to be the most efficient pancreatic enzyme in diagnosing pancreatic insufficiency (Campeotto et al., 2002). The normal FE-1 level is considered to be 200 µg/g. FE-1 levels between 100 to 200 µg/g indicate moderate pancreatic insufficiency. FE-1 levels <100 µg/g are thought to indicate severe pancreatic insufficiency. The normal adult reference level of 200 µg/g can be applied to infants that are older than two weeks, regardless of gestational age, birth weight, or nutritional status (Nissler, et al., 2001).

In the longitudinal study conducted by Campeotto et al. (2002), the FE-1 levels in term and preterm infants were collected and compared. The results showed that preterm infants had significantly lower FE-1 levels when compared to the term infants. On day 2, the results of the FE-1 levels in the term vs. preterm infants were 80 vs. 354 µg/g and on day 5 the levels were 164 vs. 600 µg/g. These results suggested pancreatic immaturity in preterm infants, especially during the first several weeks of life. The data also showed a positive correlation between an increase in FE-1 levels and total nutritional intake. This suggests that enteral feedings could be an effective strategy for increasing the maturity of the GI system at a quicker rate (Nissler et al., 2001).

In another longitudinal study, conducted by Kori et al. (2001), researchers were also able to show that term infants were able to reach normal FE-1 levels at a quicker rate than the preterm infants. While it takes an average of 3 days for term infants to reach

normal FE-1 levels, it took approximately 2 weeks for the preterm infants to achieve these levels.

While very little research has been conducted in regards to the pancreatic secretory and exocrine function of preterm infants, even less has been conducted in regards to these functions in preterm infants with BPD. Since pancreatic enzyme activity is an essential aspect of the digestive and absorptive process of nutrients and thus, for growth within this vulnerable population, further research must be conducted. Because of this, the study will examine the pancreatic functionally and exocrine capacity in these preterm infants with BPD. It is hypothesized that the preterm infants with BPD will have FE-1 levels below the normal accepted rate of 200 $\mu\text{g/g}$ of stool. It is also hypothesized that the FE-1 levels in the preterm infants will increase over time.

Chapter 3

Methodology

A. Research Design

The study's design will consist of a repeated measures study in preterm infants with BPD. BPD is defined as treatment with mechanical ventilation and/or oxygen therapy for at least the first 28 days of life. Thus, in order to be included in the study, the infant must be on oxygen at 28 days of life. The infants, with parental consent, will enter the study at 28 days of life and will be recruited from Grant and Doctors West hospitals, specifically from their Neonatal Intensive Care Units (NICU). The pancreatic enzyme activity level and growth rates of these preterm infants will be determined. The dependent variable in this study is the FE-1 level. Measures of the dependent variable will occur at 4 and 8 weeks, however, only the data from the first collection point will be reported in this study. The requirement for the preterm infants to be included in the 4th week collection point is to be at least 28 days post-birth and have been on full enteral feedings for a minimum of 72 hours. The rationale for choosing 72 hours is that preterm infants have slow and variable intestinal transit time. This will ensure that stool collected will most likely represents the full enteral nutrition that the infant received (Leitch & Denne, 2000). The 8th week collection point was chosen because by this point the calories should have been advanced to the target kcal/kg/day and the infants will have had time to establish a pattern of growth.

B. Sample

The sample consisted of 34 preterm infants that were recruited from the NICUs at Grant and Doctors West hospitals. The inclusion criteria were as follows. The preterm

infants must have had a continuous requirement for positive pressure ventilation and/or oxygen therapy at 28 days of life in order to maintain an acceptable pO₂ level. The infants must have been less than or equal to 29 weeks gestational age, appropriate for gestational age, and transitioned to full enteral nutrition. They must have been receiving human milk or preterm infant formula for the enteral feedings and the enteral feedings must have been provided as bolus feedings. The infants must have received ventilator support with oxygen supplementation during the first 72 hours of life in order to manage the respiratory distress.

The exclusion criteria were as follows. The infant was excluded from the study if they suffered from any major congenital malformations, especially respiratory, cardiac, or gastrointestinal, or if they had received any major surgery. They were excluded if there was an anoxic brain injury at birth or if any postnatal steroids had been used. They were also excluded if they were considered to be small for gestational age (birthweight < 10th percentile) or large for gestational age (> 90th percentile) because the growth patterns for these two groups are different. Infants with necrotizing enterocolitis or any malabsorption disorders were also excluded. If the infant transferred to another hospital besides Grant Hospital or Doctors West Hospital during the study, they were also excluded. Finally, the infant was excluded if they had received a soy-based or elemental formula during their enteral feedings.

C. Data Collection

Stool samples from the preterm infants were collected at approximately 28 days of life once full enteral nutrition had been established and again 4 weeks later. For this study, only data from the first collection date will be used.

D. Procedure

Preterm infants were enrolled in the study at 28 days of life if they were able to meet the required inclusion criteria. At approximately 21 days of life, the preterm infants who appeared to be meeting the inclusion criteria were identified as potential participants by the nursing staff. The nursing staff then approached the parents of the eligible preterm infants and briefly explained the research study. The nurse then requested permission from the parents to have a member of the research team contact them and explain the study in more detail. If the parents agreed, they were contacted by a member of the research team and a full explanation of the study was provided. All questions were answered and informed consent from the parents was obtained. The study then began. On study days 1 and 26, stool samples were collected over a 72 hour period and pooled together. The stool samples were collected by lining the diaper with fat-free paper, which has the ability to separate the stool from the urine. These stool samples were then used to determine the FE-1 levels of the preterm infants suffering from BPD.

Once the stool was collected and pooled for 72 hours, the FE-1 levels were determined by using an enzyme-linked immunosorbent assay (ELISA) with a monoclonal antibody (ScheBo-Biotech, USA). In preparing the stool sample for the ELISA, each sample was diluted in 10 mL of solvent and homogenized using a vibration mixer for 5 minutes. The suspension was then centrifuged and the supernatant was able to be used for analysis. The ELISA has several steps that must be completed in order to determine the FE-1 level. First, the stool supernatant was mixed with washing buffer. Then, 50 μ L of the washing buffer were pipetted into the well of the plate. The plate was then incubated for 30 minutes at room temperature and the plate was then washed. 50 μ L of anti-E1-bio-

POD-Streptavidin-Complex was added and the plate was incubated for 15 minutes at room temperature in the dark. The plate was washed again and a 100 μ l of substrate solution was added into the wells. The plate was incubated for 15 minutes at room temperature in the dark and then 100 μ l of Stop solution was added. Finally, the plate was read at OD 405 and the obtained values were then evaluated with a standard curve. In order to enhance the rigor of the study, each stool sample went through this process twice in order to ensure accurate readings.

Chapter 4

Results

The sample was comprised of 34 preterm infants with BPD who met the inclusion criteria of the larger study. All the infants were born before 29 weeks gestation and the mean gestational age of the group was 27.2 weeks gestation. The expected normal value for FE-1 is greater than 200 $\mu\text{g/g}$ of stool. The mean FE-1 levels were 287.79 $\mu\text{g/g}$ of stool with a standard deviation of ± 106.25 $\mu\text{g/g}$ of stool. The range for the FE-1 values was 101.92-520.02 $\mu\text{g/g}$ of stool. Five infants had levels below 200 $\mu\text{g/g}$ of stool.

Chapter 5

Discussion

The purpose of this study was to examine FE-1 levels in extremely preterm infants with BPD. Preterm infants with BPD have been found to excrete higher levels of calories (Boehm et al., 1996; Gallagher & Steward, unpublished data). One logical explanation is pancreatic function in relation to pancreatic enzyme activity. Previous researchers have demonstrated that preterm infants with BPD have lower pancreatic activity as evidenced by lower levels of trypsin and lipase activity (Boehm et al., 1996). The findings from the current study did not support the earlier findings by Boehm and colleagues. The current findings demonstrate that the majority of preterm infants with BPD have adequate pancreatic function by the time BPD is diagnosed at 28 days of life. There could be several potential explanations for this difference. Measurement of trypsin and lipase occurred by direct aspiration of duodenal secretions via a transpyloric tube. This allowed for a more direct assessment of pancreatic function. Although elastase-1 remains stable throughout the digestive process (Kori et al., 2003), it may be that elastase-1 levels are not as sensitive a measure as direct measure of pancreatic enzymes in duodenal secretions.

An interesting dilemma is that these data are from a larger study in which these infants excreted higher levels of calories. The reasons for this are unclear but warrant further examination since these infants are not maximizing caloric absorption. Fecal analysis of the specific contribution of each of the macronutrients (fat, carbohydrate, and protein) to fecal energy loss would shed some light on these energy losses. Researchers have demonstrated that there is a lack of correlation between FE-1 levels and fecal

nitrogen levels in stable preterm infants (Corvaglia et al., 2008). Despite the lack of a relationship, fecal nitrogen levels were higher in the preterm infants who were small for gestational age at birth.

Another important finding from this study that warrants further study is the subset of infants who had FE-1 levels below the normal value. Although not reported in this study, levels were measured at two time points, 2-4 weeks later, and were found to have increased to normal levels. It is well established that preterm infants with BPD require longer periods of time to transition from parenteral nutrition to full enteral nutrition (Khan et al., 2006). In the current study, the requirement was that the infants had made the transition to full enteral nutrition. It could be that this subset of infants required a longer period of time to make the transition. Rises in FE-1 levels are associated with the advancement of enteral nutrition (Campeotto et al., 2002). It would be interesting to follow FE-1 levels during the same period of time that the infants are making the transition to full enteral feedings.

In conclusion, discovering the reasons for the inadequate growth associated with BPD requires continual study. Based on the findings from this study, preterm infants with BPD appear to have normal pancreatic function as evidenced by expected FE-1 levels. The higher excretion of calories and the subset of infants who had lower levels at 28 days of life warrant further examination in this group of preterm infants.

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